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Yolk androgens do not appear to mediate sexual conflict over parental investment in the collared flycatcher *Ficedula albicollis*

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ABSTRACT

Males and females are in conflict over parental care, as it would be favourable for one parent to shift labour to the other. Yolk hormones may offer a mechanism through which female birds could influence offspring traits in ways that increase the relative investment by the male. We studied the role of yolk androgens in mediating sexual conflict over parental care in the collared flycatcher (*Ficedula albicollis*). In a cross-fostering experiment, the male's proportion of total feeding visits increased with increasing androgen levels in the foster eggs. This could suggest that sexual conflict over parental care may be influenced by the female's differential allocation of yolk androgens or a maternal effect associated with yolk androgens. However, when we experimentally elevated yolk androgen levels, male feeding rates did not differ between control and androgen-manipulated nests. This suggests that other egg components correlated with yolk androgen levels, rather than yolk androgen levels *per se*, may influence male parental effort. In conclusion, yolk androgens *per se* do not appear to mediate sexual conflict over parental investment in the collared flycatcher.

Introduction

In species with biparental care, females and males are in conflict over the amount of parental care they provide to their offspring (Trivers, 1972; reviewed by Arnquist and Rowe, 2005; Houston et al., 2005; Lessells, 2006; Hartley and Royle, 2007). For each parent, increased investment reduces the parent's future reproductive success and survival prospects (e.g. Williams, 1966; Roff, 1992; Parker et al., 2002), and therefore it would be favourable for one parent to shift labour to the other (Parker et al., 2002; Houston et al., 2005). The parents' decision about how much to invest in the offspring depends on offspring need and/or brood value, as well as the feeding behaviour of the other parent (e.g. Hinde and Kilner, 2007). A parent could thus try to manipulate the investment of its partner by changing either offspring traits or his or her own behaviour (e.g. Slagsvold et al., 1995; Westneat and Sargent, 1996; Arnquist and Rowe, 2005; Lessells, 2006).

Offspring phenotype is affected by maternal effects (Mousseau and Fox, 1998), for instance via egg composition (e.g. Groothuis et al., 2005a). In some bird species, androgens deposited in the egg yolk have been found to increase competitive ability of chicks (correlative data in Schwabl, 1993; experimental data in Eising et al., 2001), chick begging (experimental data: Schwabl, 1996; Schwabl and Lipar, 2002; Eising and Groothuis, 2003; Boncoraglio et al., 2006; von Engelhardt et al., 2006) and growth (experimental data: Schwabl, 1996; Eising et al., 2001; Pilz et al., 2004; Navara et al., 2005, 2006; Tschirren et al., 2005). In other studies, however, no positive effect of elevated yolk androgens on begging or growth was found (experimental data: Sockman and Schwabl, 2000; Andersson et al., 2004; Pilz et al., 2004; Uller et al., 2005; Saino et al., 2006), or the effect depended on offspring sex (e.g. Saino et al., 2006; Müller et al., 2008; Sockman et al., 2008). The effects of yolk androgens thus seem to vary, but there is evidence that they can affect offspring traits. It has therefore been proposed that by allocating more yolk androgens into the egg, a female could affect offspring trait(s) to which males respond, and this way increase the relative feeding effort of the male to her benefit (Groothuis et al., 2005a; Michl et al., 2005; Gil et al., 2006; Lessells, 2006; Moreno-Rueda, 2007; Müller et al., 2007a).

This hypothesis relies on several assumptions. First, yolk androgen levels should affect chick traits to which the parents respond by changing their food provisioning. There is evidence from several species for this assumption (reviewed e.g. by Müller et al., 2007a, see

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above). However, a direct link between yolk androgen levels and parental feeding effort has been investigated in only one study thus far (Tschirren and Richner, 2008). Second, increasing yolk androgen levels should either impose a cost or be constrained, since otherwise all females could afford to allocate high levels of androgens (e.g. Groothuis et al., 2005a; Groothuis and Schwabl, 2008). Indeed, elevated levels of androgens in the yolk can be costly for the chicks, as an immunosuppressive effect of androgens has been reported in some studies (e.g. Groothuis et al., 2005b; Müller et al., 2005; Navara et al., 2005; but see Andersson et al. 2004; Tschirren et al., 2005; Müller et al., 2007b). Moreover, such elevated levels may require elevated circulating androgen levels in the female, which may, for example, delay or inhibit egg laying (e.g. Clotfelter et al., 2004; Rutkowska et al., 2005; discussed in Groothuis and Schwabl, 2008). Finally, females should be able to respond differently to the chick trait influenced by yolk androgens than males. This assumption is yet to be explored. Two mechanisms are possible, depending on whether both parents or only the male respond to the trait through which the female attempts to manipulate the male (Müller et al., 2007a). 1) If both parents respond to the androgen-mediated offspring trait, males are expected to increase their investment in response to increasing yolk androgen levels deposited in the eggs by the female; females, however, should have lower responsiveness to androgen-mediated chick traits when allocating high androgen levels into their eggs than when allocating low levels. 2) If only males respond to the chick trait (hereafter a *male-specific* trait), males should increase their feeding rate with increasing yolk androgen levels while females should not change their investment. Previous studies have shown that it is possible that females and males respond to different components of nestling behaviour and phenotype, for example colour (Jourdie et al. 2004, de Ayala et al. 2007; Ewen et al. 2008; Galvan et al. 2008; Tanner and Richner, 2008), size (reviewed in Slagsvold, 1997; Lessells, 2002), condition (e.g. Christe et al. 1996) or visual vs. vocal cues of begging (Kilner, 2002, reviewed in Müller et al., 2007a).

We studied whether yolk androgens could mediate sexual conflict over parental care in a small passerine bird, the collared flycatcher (*Ficedula albicollis*) by conducting two experiments. Firstly, we examined whether naturally varying levels of yolk androgens affect the relative work load of the parents. We did this by cross-fostering full broods with naturally varying yolk androgen levels among nests and monitoring the subsequent parental feeding rates. We predicted that if females can manipulate male investment through differential yolk androgen deposition, and the androgen-mediated nestling trait is male-specific, the proportion of male feeding visits should be positively correlated with the yolk androgen levels of the foster eggs (hereafter foster yolk androgens), but not of original eggs (hereafter original yolk androgens). In contrast, if the manipulation is mediated through an androgen-mediated trait to which both parents respond, we predicted a positive correlation between proportion of male feeding visits and original androgen level, but no correlation with proportion of male feeding visits and foster androgen level. This is because females allocating high yolk androgen levels should have lower responsiveness to androgen-mediated chick traits than females allocating low yolk androgen levels. Secondly, we experimentally increased yolk androgen levels of whole clutches and monitored the subsequent parental feeding rates to test whether yolk androgens *per se* allow females to manipulate males. We predicted that if male manipulation by the female is mediated by yolk androgens *per se*, male feeding rate should be higher in broods of the androgen-manipulated than the control group.

Methods

Study site and study species

The experiments were conducted in Spring 2007 on the island of Gotland, Sweden (57°19' N, 18°29' E) in a nest box breeding population of collared flycatchers monitored since 1980 (Gustafsson, 1989). The study area consists of several small and spatially discrete forest patches. The collared flycatcher is a small (ca. 13 g), migratory passerine, which breeds in Central and Eastern Europe and on the islands of Gotland and Öland in Sweden. Average clutch size in this population is six eggs, and average brood size four nestlings. Usually both parents feed the young, but polygynous males (ca. 10%) mainly feed their primary brood (Gustafsson, 1989; Gustafsson and Qvarnström, 2006).

The cross-fostering experiment

Nest boxes were checked every other day starting at end of April to monitor the progress of nest building and egg laying. When one or two eggs were found in the nest, they were marked with non-toxic permanent marker and the nest box was visited the in following day (s) to collect the third egg of the clutch on the day of laying ($N = 31$ nests). These eggs were taken to the laboratory, and yolks were separated from albumen on the same day and stored frozen at −20 °C until hormone analysis (see below). The collected egg was replaced by a dummy egg in each nest. The average clutch size in this species and population is six eggs, so the third egg is one of the middle eggs of a clutch. In most species studied so far, including the collared flycatcher and its sister species the pied flycatcher (*Ficedula hypoleuca*), the between-clutch variation in yolk androgen levels is higher than the within-clutch variation (e.g. Reed and Vleck, 2001; Groothuis and Schwabl, 2002; Pilz et al., 2003; Tschirren et al., 2004; Michl et al., 2005; Müller et al., 2007b, Tobler et al., 2007; T. Laaksonen, unpubl. data). No within-clutch pattern of yolk androgens was found in a Hungarian population of the collared flycatchers (Michl et al., 2005), thus yolk androgen concentration in the middle egg should be a good proxy for the mean hormone level of a clutch. Even if within-clutch variation in hormone deposition would have occurred in some individuals of our study population, the third egg would still represent the medium concentration of the whole clutch, as long as the pattern of within-clutch variation is linear (as found in many studied species with significant within-clutch variation, e.g. Reed and Vleck, 2001; Groothuis and Schwabl, 2002; Pilz et al., 2003; Tschirren et al., 2004).

Nests were monitored to record final clutch size and hatching date as part of the population monitoring. Two days after hatching, complete broods were cross-fostered between sampled nests (matched for hatching date and brood size), so that in most cases the yolk androgen levels of the foster brood (i.e. of the third egg of the foster brood) was known. Both parents were caught in the nest box using a swing-door-trap when feeding 6 to 10 day-old nestlings. They were weighed and measured and their age was determined as yearling or older (see e.g. Pärt and Gustafsson, 1989).

Parental feeding frequency was measured at day 6 after hatching by observing parental feeding behaviour from a hide ca. 15 m distance from the nest. After two feeding visits by the parents (to ensure that they were feeding normally), 1 h of feeding visits by the parents was observed. Peak of feeding activity in this species occurs between day 5 and day 10 of nestling rearing. Feeding rate was measured as the number of feeding visits by each parent per hour. Feeding rate has been shown to correlate positively with energy expenditure in this population (Pärt et al., 1992), thus it can be used as a measure of parental investment. Parental feeding rate further correlates with both chick weight and condition at fledging and fledging success (Doligez et al., 2004), and these variables are related to juvenile survival in this species (Linden et al., 1992). Feeding rates were recorded mostly between 8.00–12.00 am, and only in good weather conditions.

Yolk androgen analysis

Yolk testosterone (T) and androstenedione (A4) concentrations were analysed by radioimmunoassay (RIA). Yolks were thawed and

homogenised with 400 µl of distilled water. Aliquots of this yolk/water emulsion (approximately 100 mg) were mixed with 150 µl of distilled water and 50 µl of 3H Tracer T (ca. 2000 counts per minute) to assess extraction efficiency. The samples were extracted twice with 2.5 ml of 70% diethylether/30% petroleumether (vol: vol) and dried under a stream of nitrogen. The extracts were then re-dissolved in 1 ml 70% methanol, centrifuged and decanted. The supernatant was dried under a stream of nitrogen and re-dissolved in PBS. T and A4 were measured in duplicates using DSL (Diagnostic System Laboratories, USA) radioimmunoassay kits following the manufacturer's protocol. The average recovery rate was 72%. The yolks were analysed in two assays with an inter-assay coefficient of variation of 5.9% for A4 and 1.6% for T and an intra-assay coefficient of variation of 10.0% for A4 and 5.7% for T. Parallelism was confirmed, and concentrations of T and A4 were not affected by extraction efficiency (T concentration: $F_{1,21}=0.01$, $p=0.94$; A4 concentration: $F_{1,21}=0.0$, $p=0.99$; range of extraction efficiency: 66.7–76.6%), egg weight (T: $F_{1,21}=0.02$, $p=0.89$; A4: $F_{1,21}=0.01$, $p=0.92$), yolk weight (T: $F_{1,21}=0.69$, $p=0.42$; A4: $F_{1,21}=0.06$, $p=0.80$) or assay (T: $F_{1,21}=1.70$, $p=0.21$; A4: $F_{1,21}=3.73$, $p=0.07$). There was no correlation between original and foster yolk androgen levels ($r=-0.31$, $N=19$, $p=0.19$). Original yolk androgen levels of eggs from 26 nests could be analysed, but due to problems in cross-fostering, both original and foster androgen levels (as estimated from the third egg) could be obtained for 19 nests only. We decided to use the sum of the concentrations of A4 and T (total androgen concentration, pg/mg) in the analyses as a measure of total androgen available for binding to either androgen or oestrogen receptors. This method was chosen because A4 is the total source (precursor) available for hormone conversion to active androgens [testosterone and dihydrotestosterone, (DHT)] and estradiol (E2), T is also a source for DHT and E2, and DHT has a higher affinity to androgen receptors than T (reviewed by e.g. Norris, 1996, Groothuis and Schwabl, 2008). In addition, A4 and T levels are positively correlated ($r=0.50$, $N=26$, $p=0.01$). However, whether the effects of the two hormones differ is uncertain, and their affinity to receptors is likely to differ (e.g. Groothuis and Schwabl, 2008). Therefore, we also examined the effects of each hormone separately and report the results when they clearly differ for the two hormones.

Yolk androgen manipulation experiment

This experiment was conducted on a different set of nests ($N=66$) than the cross-fostering experiment. Nest boxes were checked every other day to determine laying dates. On the estimated day of clutch completion (i.e. day of laying of the sixth egg), each clutch was randomly assigned to either the control or the androgen-manipulation group. All the eggs of a clutch were treated in the same way. The eggs were replaced with dummy eggs for the time of the injections. In the androgen-manipulation group ($N=36$ nests), the eggs were injected with 14.4 ng of testosterone (Fluka) and 50.8 ng of androstenedione (Fluka) dissolved in 4 µl sesame oil. In the control group ($N=30$ nests), eggs were injected only with 4 µl of sesame oil. The amount of injected androgens was calculated using previous data on natural yolk androgen levels from the same population (T mean: 14.2 ng/yolk, SE 0.4 ng/yolk, maximum: 28.8 ng/yolk; A4 mean: 60.3 ng/yolk, SE 1.5 ng/yolk, maximum 111.1 ng/yolk; B. Doligez and B. Tschirren, unpubl. data). The amount injected corresponds to the difference between mean and maximum values of androgens per yolk, which ensured that final yolk androgen level in the androgen-manipulated clutches was on average at the upper limit of the natural range. The original androgen concentration in the manipulated clutches was not determined, as this would have required removing an egg and thus changing brood size, which could have had a substantial effect on the parental feeding rates. The position of the yolk was visualized using a light source positioned beneath the egg. The surface of the egg was first cleaned with 95% ethanol and a small hole was made using a disposable 27 G needle. The oil-vehicle was injected into the yolk using a 25 µl Hamilton syringe (702RN) and 26 G needle. After the injection, the hole in the egg shell was sealed with a drop of tissue adhesive (Vet-Seal, B. Braun Medical, Switzerland) or with a patch of a flexible wound film (Opsite, Smith & Nephew, UK). The eggs were returned to the nest immediately after the injections. Nests were checked on the following day and the seventh egg was injected if present, according to the clutch treatment. This method for manipulating yolk androgen levels has been successfully used and validated in previous studies (e.g. Tschirren et al., 2005). The hatching success of injected eggs did not differ between the control group (79.74%) and the androgen-manipulated group (79.77%, Wilcoxon test: $p=0.94$, $N=66$). The natural hatching success of non-injected clutches (excluding deserted and predated nests, where no egg hatched) in this population is 92.0% (unmanipulated nests of years 1997–2002, $N=1677$, B. Doligez and L. Gustafsson, unpubl. data)

On day 9 after hatching, parental feeding rates were recorded with digital video cameras for 2 h (SONY Handycam DCR-SR52). If parents were giving frequent alarm calls at the beginning of the recording, feeding rates were estimated from the time when parents stopped alarming and made the first visit back to the nest. Video recordings were watched blindly, i.e. the observers were unaware of nest treatment. As for the cross-fostering experiment, parents were caught, weighed and their age was determined. Collared flycatchers are partly polygynous (ca. 10% in this population, Gustafsson, 1989; Gustafsson and Qvarnström, 2006), but none of the males in either experiment were recorded feeding at a secondary nest, and thus polygyny does not affect our results.

We tested whether feeding data from the two experiments obtained at different nestling ages (6 and 9 days for the cross-fostering and injection experiment, respectively) are comparable by analysing both 6 days and 9 days post hatching feeding rates in a sample of nests. There was a significant, positive correlation in the male share of feedings between day 6 and day 9 ($r_s=0.67$, $p=0.0003$, $N=25$), and the average male share of feedings was equal at these two measuring points (average male share of feedings: day 6 = 49.4%; day 9 = 49.0%), which strongly indicates that the division of the labour within a pair is fairly constant over this 3-day feeding period.

All experiments were conducted under licences from the Swedish National Board for Laboratory Animals and the Bird Ringing Centre of the Swedish Museum of Natural History (Stockholm, Sweden). The experimental protocols adhered to the standards of US National Institute of Health.

Statistical analysis

All statistical analyses were performed with SAS 9.0. Nests where only one parent was observed feeding, or where the age of one parent was unknown (some males could not be caught) or uncertain, were not used in the analyses, which reduces the sample size.

In the cross-fostering experiment, we analysed the male's proportion of the total number of feeding visits in relation to yolk androgen concentration (as estimated from the third egg) in original and foster eggs, using a general linear model. We also included female and male age as explanatory variables to control for possible age effects. Laying date, brood size and time of the day when the observations were started were added as covariates in this as well as in all subsequent models. In a second step, we included egg mass and yolk mass in the model to test whether they were associated with male share of feeding activity. The interaction between female and male age could not be tested due to small sample size in the yearling female–yearling male category (sample sizes: yearling females = 3, older females = 13, yearling males = 2, older males = 14).

In the androgen-manipulation experiment, we analysed parental feeding rates in relation to androgen-manipulation treatment using a general linear mixed model. We tested the influence of treatment

(control or androgen-manipulated), sex, age of the parent and their interactions on the number of feeding visits per hour (i.e. feeding rate). Sample sizes were: yearling females = 12, older females = 32, yearling males = 14, older males = 30. Nest identity (nested within treatment) and forest patch were included as random effects to control for the non-independence of parental responses within a pair and for patch effects.

We used a backward model selection procedure, removing interactions, covariates and main effects starting with the least significant. To confirm the non-significance of the removed interaction terms and main effects, each term was added to the final model separately. Satterthwaite approximation was used to determine denominator degrees of freedom (Littell et al., 1996) and normality of the residuals was checked.

Results

Male feeding rate in the cross-fostering experiment

Average female feeding rate (±SD) was 12.0±5.2 feeding visits/hour ($N=31$) and average male feeding rate was 11.4±4.5 ($N=28$). The propor-tion of feeding visits by the male increased with increasing yolk andro-gen levels in the foster eggs (as measured from the third egg; $F_{1,12}=8.98$, $p=0.011$, Fig. 1, average proportion of male feedings (±SD): 49.4±17.9%), controlling for laying date ($F_{1,12}=10.17$, $p=0.008$, $\beta\pm SE=-0.037\pm0.011$), and time of the day ($F_{1,12}=4.38$, $p=0.058$; $\beta\pm SE=0.031\pm0.015$). The proportion of feeding visits by the male was not related to the original yolk androgen levels ($F_{1,11}=0.24$, $p=0.63$). Separate analyses of both androgens revealed that male proportion of feeding visits was related to yolk A4 ($F_{1,12}=12.33$, $p=0.004$) but not to yolk testosterone levels ($F_{1,12}=0.07$, $p=0.79$). In order to find out whether the increase in the proportion of male feeding visits was explained by an increase in male feeding rate or a decrease in female feeding rate, we analysed the absolute feeding rates of both sexes. Male feeding rate increased with increasing foster yolk androgen concentration ($r=0.45$, $N=20$, $p=0.042$), whereas no significant correlation was observed in females ($r=-0.24$, $N=20$, $p=0.30$). There was an interaction between parental sex and fos-ter yolk androgens ($F_{1,28}=4.26$, $p=0.048$; males: $\beta\pm SE=0.033\pm0.028$; females: $\beta\pm SE=-0.028\pm0.050$). These results are in accordance with the hypothesis that females manipulate males through a yolk androgen-dependent nestling signal, to which only males react, and/or a maternal effect that correlates with yolk androgen concentrations in the eggs. Age of the parents did not affect the proportion of feeding visits by the male, and there were no significant interactions between female or male age and original or foster yolk androgen concentration (all p-values>0.08). Finally, the proportion of feeding visits by the male was not related to egg mass ($F_{1,11}=0.41$, $p=0.53$) or yolk mass ($F_{1,11}=0.32$, $p=0.58$). Thus the relationship between yolk androgen content in foster eggs and male feeding effort can not be explained by egg or yolk mass.

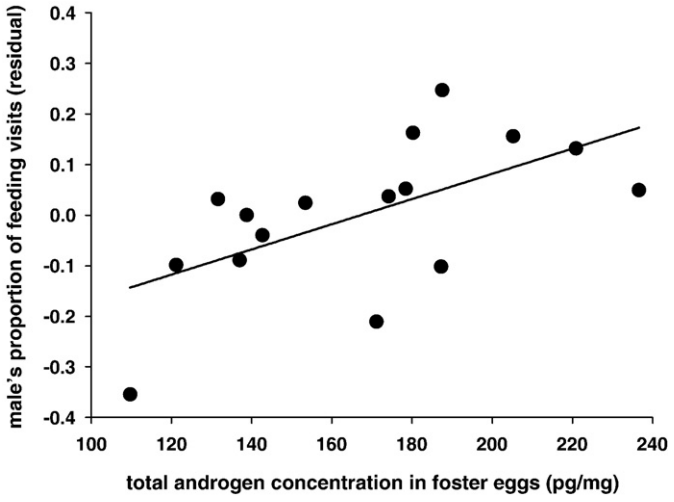

Fig. 1. Male's proportion of feeding visits in relation to total androgen concentration in eggs (as estimated from the third egg of the clutch) of fostered nestlings ($N=16$, $F_{1,12}=8.98$, $p=0.011$). Residuals are from a model in which the effects of laying date and time were removed.

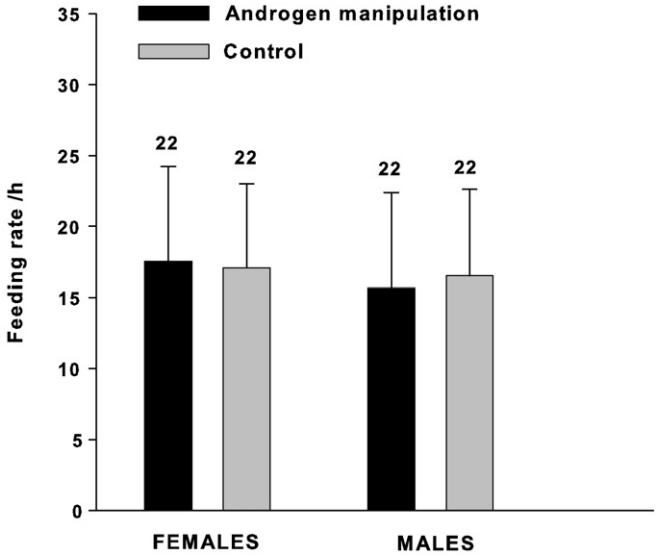

Fig. 2. Male and female feeding rates (mean, SD) in control and androgen manipulated nests (interaction treatment×sex: $F_{1,42}=0.46$, $p=0.50$). Sample size is indicated above the bars.

Feeding rates in the yolk androgen-manipulation experiment

There was no effect of the androgen-manipulation on parental feeding rates and no interaction between treatment and sex (interaction treatment×sex: $F_{1,42}=0.46$, $p=0.50$; treatment: $F_{1,41}=0.38$, $p=0.82$; sex: $F_{1,42}=1.56$, $p=0.21$, Fig. 2.). Average female feeding rate (±SD) was 17.3±6.2 feeding visits/hour ($N=44$) and average male feeding rate was 16.7±6.3 ($N=44$) (average proportion of male feedings (±SD) being: 48.7±11.3%). Feeding rates of yearling and older individuals did not differ between androgen-manipulated and control group (interaction age×treatment: $F_{1,82.6}=0.12$, $p=0.73$; age: $F_{1,82.3}=0.02$, $p=0.89$). Feeding rate increased with brood size (controlled for in the above analyses; $F_{1,37}=13.87$, $p<0.001$, $\beta\pm SE=2.336\pm0.627$). All other interactions and covariates were non-significant (all p-values>0.12).

Discussion

Do females manipulate males through yolk androgens?

The results of the cross-fostering experiment suggest that, in the collared flycatcher, sexual conflict over parental care may be influenced by the female via differential allocation of yolk androgens or other egg components (e.g. carotenoids, lipids) that are correlated with the yolk androgen content of the egg. The correlation between the proportion of feeding visits by the male and yolk androgens in the foster eggs was explained by an increase in male absolute feeding rate, which suggests that offspring traits associated with high yolk androgen levels make specifically males to increase their effort. However, when yolk androgen levels were experimentally elevated, males feeding nestlings originating from androgen-manipulated eggs

did not feed at a higher rate than those feeding control clutches. This indicates that the androgen levels *per se* did not affect male effort.

It could be argued that the effects of androgens on the chicks may depend on synergistic interactions with other egg components (e.g. Groothuis and von Engelhardt, 2005), or that exogenous A4 and T in the yolk could be differentially metabolized by steroidogenic enzymes than endogenous steroids. In this case, experimental elevation of only the androgen levels using exogenous hormones might not have had an effect on the chicks. This is however unlikely, because experimental elevation of yolk androgen levels following the same protocol as ours had a sex-specific effect on chick growth in another set of nests in our study population (Pitala et al., in press). This shows that exogenous androgens do have an effect on the chicks. Thus the result of the androgen-manipulation allows us to conclude that yolk androgens do not appear to mediate sexual conflict over parental care in the collared flycatcher (potential explanations for the association between yolk androgens and male investment in the cross-fostering experiment are discussed below).

To our knowledge, only one study thus far has examined whether yolk androgens may play a role in mediating sexual conflict over parental investment. In great tits, only females adjusted their parental effort to androgen-mediated nestling traits, indicating that male manipulation by the female via yolk androgens is also unlikely in this species (Tschirren and Richner, 2008). Thus no empirical evidence currently supports the hypothesis that females could successfully manipulate male investment through yolk androgens. Selection may have favoured males' reduced susceptibility to yolk androgen-mediated nestling traits, allowing them to resist the manipulation by females (Tschirren and Richner, 2008). In an evolutionary conflict of interests, such as sexual conflict over parental investment, an "arms-race" is expected, with each parent trying to minimise costs and maximise benefits (e.g. Arnquist and Rowe, 2005). Thus males may be expected to evolve responses to specific offspring signals that cannot be manipulated by females and/or ignore signals that can be manipulated by females (Müller et al., 2007a). Indeed, males and females are known to use different cues, for example nestling sex, size, position, vocal or visual components of begging, in adjusting their feeding effort (e.g. reviewed in Lessells, 2002, Müller et al., 2007a). The evolution of male counter-responses to female manipulation will depend on the balance between benefits (avoiding manipulation by the female) and costs (e.g. reduced reproductive success) of ignoring the manipulated nestling traits. Unfortunately, empirical testing of the hypothesis that past manipulation by females has selected males to ignore certain offspring signals may prove very difficult. However, Lessells (2006) pointed out that, in general, manipulative behaviour in sexual conflict over parental investment may be rare, as the benefits may be small and the costs could be substantial. Thus, sexual conflict over parental investment may not generate rapid evolutionary change and antagonistic coevolution.

Relationship between male investment and yolk androgens in the cross-fostering experiment

The results of the androgen manipulation experiment suggest that yolk androgens *per se* do not mediate sexual conflict over parental investment. Therefore, the correlation between male share of parental care and yolk androgen level of fostered clutches in the cross-fostering experiment (Fig. 1) probably arises through a different pathway. For instance, the amount of yolk androgens in the egg could be correlated with another egg component that females could use to manipulate male effort. The amount of yolk androgens has indeed been found to correlate with, for example, the amounts of antioxidants and immunoglobulins in the yolk (e.g. Royle et al., 2001; Groothuis et al., 2006; Török et al., 2007). The role of egg components other than androgens in mediating sexual conflict over parental investment however remains to be investigated. The amount of yolk androgens could also be positively correlated with the genetic quality or condition of the parents and subsequently with the quality of the offspring, to which the parents respond. It is yet possible that the quality of the offspring depends on the quality of their natal territory. A correlation with yolk androgen levels could then arise for example if higher quality territories are more fiercely defended, leading to higher androgen levels in female circulation and subsequently to higher yolk androgen levels in the eggs (e.g. Schwabl, 1997). Thus the positive correlation between male parental care and yolk androgens could arise from males responding to the overall quality of the offspring.

Conclusions

The results of our experiments indicate that female collared flycatchers in our population do not differentially allocate yolk androgens to manipulate males into higher parental investment. However, they might be able to manipulate males via other maternal effects correlated with yolk androgens. Identifying the egg components or chick characteristics that appear to differentially influence the effort of male and female parents would be an important next step towards a better understanding of the potential of maternal effects in mediating sexual conflict over parental investment.

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References

Andersson, S., Uller, T., Lõhmus, M., Sundström, F., 2004. Effects of egg yolk testosterone on growth and immunity in a precocial bird. J. Evol. Biol. 17, 501–506.
Arnquist, G., Rowe, L., 2005. Sexual Conflict. Princeton University press, Princeton.
Boncoraglio, G., Rubolini, D., Romano, M., Martinelli, R., Saino, N., 2006. Effects of elevated yolk androgens on perinatal begging behavior in yellow-legged gull (*Larus michahellis*) chicks. Horm. Behav. 50, 442–447.
Christe, P., Richner, H., Oppliger, A., 1996. Begging, food provisioning, and nestling competition in great tit broods infested with ectoparasites. Behav. Ecol. 7, 127–131.
Clotfelter, E.D., O'Neal, D.M., Gaudioso, J.M., Casto, J.M., Parker-Reng, I.M., Snajdr, E.A., Duffy, D.L., Nolan Jr., V., Ketterson, E.D., 2004. Consequences of elevating plasma testosterone in females of a socially monogamous songbird: evidence of constraints on male evolution? Horm. Behav. 46, 171–178.
de Ayala, R.M., Saino, N., Møller, A.P., Anselmi, C., 2007. Mouth coloration of nestlings covaries with offspring quality and influences parental feeding behaviour. Behav. Ecol. 18, 526–534.
Doligez, B., Pärt, T., Danchin, E., 2004. Prospecting in the collared flycatcher: gathering public information for future breeding habitat selection? Anim. Behav. 67, 457–466.
Eising, C.M., Groothuis, T.G.G., 2003. Yolk androgens and begging behavior in black-headed gull chicks: an experimental field study. Anim. Behav. 66, 1027–1034.
Eising, C.M., Eikenaar, C., Schwabl, H., Groothuis, T.G.G., 2001. Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: consequences for chick development. Proc. R. Soc. Lond. B. 268, 839–846.
Ewen, J.G., Thorogood, R., Karadas, F., Cassey, P., 2008. Condition dependence of nestling mouth colour and the effect of supplementing carotenoids on parental behaviour in the hihi (*Notiomystis cincta*). Oecologia 157, 361–368.
Galvan, I., Amo, L., Sanz, J.J., 2008. Ultraviolet–blue reflectance of some nestling plumage patches mediates parental favouritism in great tits *Parus major*. J. Avian Biol. 39, 277–282.
Gil, D., Ninni, P., Lacroix, A., de Lope, F., Tirard, C., Marzal, A., Møller, A.P., 2006. Yolk androgens in the barn swallow (*Hirundo rustica*): a test of some adaptive hypotheses. J. Evol. Biol. 19, 123–131.
Groothuis, T.G.G., Schwabl, H., 2002. Determinants of within- and among-clutch variation in levels of maternal hormones in black-headed gull eggs. Funct. Ecol. 16, 281–289.

Groothuis, T.G.G., von Engelhardt, N., 2005. Investigating maternal hormones in avian eggs: measurement, manipulation and interpretation. Ann. NY Acad. Sci. 1046, 168–180.
Groothuis, T.G.G., Schwabl, H., 2008. Hormone-mediated maternal effects in birds: mechanisms matter but what do we know of them? Phil. Trans. R. Soc. B. 363, 1647–1661.
Groothuis, T.G.G., Müller, W., von Engelhardt, N., Carere, C., Eising, C.M., 2005a. Maternal hormones as a tool to adjust offspring phenotype in avian species. Neurosci. Biobehav. Rev. 29, 329–352.
Groothuis, T.G.G., Eising, C.M., Dijkstra, C., Müller, W., 2005b. Balancing between costs and benefits of maternal hormone deposition in avian eggs. Biol. Lett. 1, 178–181.
Groothuis, T.G.G., Eising, C., Blount, J.D., Surai, P., Apanius, V., Dijkstra, C., Müller, W., 2006. Multiple pathways of maternal effects in black-headed gull eggs: constraint and adaptive compensatory adjustment. J. Evol. Biol. 19, 1304–1313.
Gustafsson, L., 1989. Collared flycatcher. In: Newton, I. (Ed.), Lifetime Reproduction in Birds. Academic Press, London, pp. 75–88.
Gustafsson, L., Qvarnström, A., 2006. A test of the "sexy son" hypothesis: sons of polygynous collared flycatchers do not inherit their fathers' mating status. Am. Nat. 167, 297–302.
Hartley, I.R., Royle, N.J., 2007. Sexual conflict and its implications for fitness. In: Jamieson, B.G.M. (Ed.), Reproductive Biology and Phylogeny of Birds. Science Publishers, Enfield, pp. 239–269.
Hinde, C.A., Kilner, R.M., 2007. Negotiations within the family over the supply of parental care. Proc. R. Soc. Lond. B 274, 53–60.
Houston, A.I., Székely, T., McNamara, J.M., 2005. Conflict between parents over care. Trends Ecol. Evol. 20, 33–38.
Jourdie, V., Benoît, M., Bennett, A.T.D., Heeb, P., 2004. Ultraviolet reflectance by the skin of nestlings. Nature 431, 262.
Kilner, R.M., 2002. Evolution of complex begging displays. In: Wright, J., Leonard, M.L. (Eds.), The Evolution of Begging, Competition, Cooperation and Communication. Kluwer Academic Publishers, Dordrecht, pp. 87–106.
Lessells, C.M., 2002. Parentally biased favouritism: why should parents specialize in caring for different offspring? Proc. R. Soc. B 357, 381–403.
Lessells, C.M., 2006. The evolutionary outcome of sexual conflict. Phil. Trans. R. Soc. Lond. B 361, 301–317.
Linden, M., Gustafsson, L., Pärt, T., 1992. Selection on fledging mass in the collared flycatcher and the great tit. Ecology 73, 336–343.
Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., 1996. SAS System for Mixed Models. SAS Institute Inc., Cary.
Michl, G., Török, J., Péczely, P., Garamszegi, L.Z., Schwabl, H., 2005. Female collared flycatchers adjust yolk testosterone to male age, but not to attractiveness. Behav. Ecol. 16, 383–388.
Moreno-Rueda, G., 2007. Yolk androgen deposition as a female tactic to manipulate paternal contribution. Behav. Ecol. 18, 496–498.
Mousseau, T.A., Fox, C.W., 1998. The adaptive significance of maternal effects. Trends Ecol. Evol. 13, 403–407.
Müller, W., Groothuis, T.G.G., Kasprzik, A., Dijkstra, C., Alatalo, R.V., Siitari, H., 2005. Prenatal androgen exposure modulates cellular and humoral immune function of black-headed gull chicks. Proc. R. Soc. Lond. B 272, 1971–1977.
Müller, W., Lessells, C.M., Korsten, P., von Engelhardt, N., 2007a. Manipulative signals in family conflict? On the function of maternal yolk hormones in birds. Am. Nat. 169, E84–E96.
Müller, W., Deptuch, K., López-Rull, I., Gil, D., 2007b. Elevated yolk androgen levels benefit offspring development in a between-clutch context. Behav. Ecol. 18, 929–936.
Müller, W., Vergauwen, J., Eens, M., 2008. Yolk testosterone, postnatal growth and song in male canaries. Horm. Behav. 54, 125–133.
Navara, K.J., Hill, G.E., Mendonça, M.T., 2005. Variable effects of yolk androgens on growth, survival, and immunity in eastern bluebird nestlings. Physiol. Biochem. Zool. 78, 570–578.
Navara, K.J., Hill, G.E., Mendonça, M.T., 2006. Yolk testosterone stimulates growth and immunity in house finch chicks. Phys. Biochem. Zool. 97, 550–555.
Norris, D.O., 1996. Vertebrate Endocrinology. Academic Press, San Diego.
Parker, G.A., Royle, N.J., Hartley, I.R., 2002. Intrafamilial conflict and parental investment: a synthesis. Phil. Trans. R. Soc. Lond. B. 357, 295–307.
Pilz, K.M., Smith, H.G., Sandell, M.I., Schwabl, H., 2003. Interfemale variation in egg yolk androgen allocation in the European starling: do high-quality females invest more? Anim. Behav. 65, 841–850.
Pilz, K.M., Quiroga, M., Schwabl, H., Adkins-Regan, E., 2004. European starling chicks benefit from high yolk testosterone levels during a drought year. Horm. Behav. 46, 179–192.
Pitala, N., Ruuskanen, S., Laaksonen, T., Doligez, B., Tschirren, B., Gustafsson, L., in press. The effects of experimentally manipulated yolk androgens on growth and immune function of male and female nestling collared flycatchers. J. Avian Biol.
Pärt, T., Gustafsson, L., 1989. Breeding dispersal in the collared flycatcher (*Ficedula albicollis*): Possible causes and reproductive consequences. J. Anim. Ecol. 58, 305–320.
Pärt, T., Gustafsson, L., Moreno, J., 1992. "Terminal investment" and sexual conflict in the collared flycatcher (*Ficedula albicollis*). Am. Nat. 140, 868–882.
Reed, W.L., Vleck, C.M., 2001. Functional significance of variation in egg-yolk androgens in the American coot. Oecologia 128, 164–171.
Roff, D.A., 1992. The Evolution of Life Histories. Oxford University Press, Oxford.
Royle, N.J., Surai, P.F., Hartley, I.R., 2001. Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects? Behav. Ecol. 12, 381–385.
Rutkowska, J., Cichoń, M., Puerta, M., Gil, D., 2005. Negative effects of elevated testosterone on female fecundity in zebra finches. Horm. Behav. 47, 585–591.
Saino, N., Ferrari, R.P., Romano, M., Lacroix, A., Gil, D., Møller, A.P., 2006. Maternal allocation of androgens and antagonistic effects of yolk androgens on sons and daughters. Behav. Ecol. 17, 172–181.
Schwabl, H., 1993. Yolk is a source of maternal testosterone for developing birds. Proc. Natl. Acad. Sci. U. S. A. 90, 11446–11450.
Schwabl, H., 1996. Maternal testosterone in the avian egg enhances postnatal growth. Comp. Biochem. Physiol. 114, 271–276.
Schwabl, H., 1997. The contents of maternal testosterone in house sparrow *Passer domesticus* eggs vary with breeding conditions. Naturwissenschaften 84, 406–408.
Schwabl, H., Lipar, J., 2002. Hormonal regulation of begging behaviour. In: Wright, I., Leonard, M.L. (Eds.), The Evolution of Begging: Competition, Cooperation and Communication. Kluwer Academic Publishers, Dordrecht, pp. 221–244.
Slagsvold, T., 1997. Brood division in birds in relation to offspring size: sibling rivalry and parental control. Anim. Behav. 54, 1357–1368.
Slagsvold, T., Amundsen, T., Dale, S., 1995. Costs and benefits of asynchronous hatching in blue tits *Parus caeruleus*. J. Anim. Ecol. 64, 563–578.
Sockman, K.W., Schwabl, H., 2000. Yolk androgens reduce offspring survival. Proc. R. Soc. Lond. B 267, 1451–1456.
Sockman, K.W., Weiss, J., Webster, M.S., Talbott, V., Schwabl, H., 2008. Sex-specific effects of yolk-androgens on growth of nestling American kestrels. Behav. Ecol. Sociobiol. 62, 617–625.
Tanner, M., Richner, H., 2008. Ultraviolet reflectance of plumage for parent–offspring communication in the great tit (*Parus major*). Behav. Ecol. 19, 369–373.
Tobler, M., Granblom, M., Sandell, M., 2007. Maternal androgens in the pied flycatcher: timing of breeding and within-female consistency. Oecologia 151, 731–760.
Trivers, R.L., 1972. Parental investment and sexual selection. In: Campbell, B. (Ed.), Sexual Selection and the Descent of Man. Aldine Press, Chicago, pp. 136–179.
Tschirren, B., Richner, H., 2008. Differential effects of yolk hormones on maternal and paternal contribution to parental care. Anim. Behav. 75, 1989–1994.
Tschirren, B., Richner, H., Schwabl, H., 2004. Ectoparasite-modulated deposition of maternal androgens in great tit eggs. Proc. R. Soc. Lond. B 271, 1371–1375.
Tschirren, B., Saladin, V., Fitze, P.S., Schwabl, H., Richner, H., 2005. Maternal yolk testosterone does not modulate parasite susceptibility or immune function in great tit nestlings. J. Anim. Ecol. 74, 675–682.
Török, J., Hargitai, R., Hegyi, G., Matus, Z., Michl, G., Péczely, P., Rosivall, B., Tóth, G., 2007. Carotenoids in the egg yolks of collared flycatchers (*Ficedula albicollis*) in relation to parental quality, environmental factors and laying order. Behav. Ecol. Sociobiol. 61, 541–550.
Uller, T., Eklöf, J., Andersson, S., 2005. Female egg investment in relation to male sexual traits and the potential for transgenerational effects in sexual selection. Behav. Ecol. Sociobiol. 57, 584–590.
von Engelhardt, N., Carere, C., Dijkstra, C., Groothuis, T.G.G., 2006. Sex-specific effect of yolk testosterone on survival, begging and growth of zebra finches. Proc. R. Soc. Lond. B 273, 65–70.
Westneat, D.F., Sargent, R.C., 1996. Sex and parenting: the effects of sexual conflict and parentage on parental strategies. Trends Ecol. Evol. 11, A87–A91.
Williams, G.C., 1966. Natural selection, costs of reproduction, and refinement of Lack's principle. Am. Nat. 100, 687–690.